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The invasion of southern South America by imported bumblebees and associated parasites

Schmid-Hempel, Regula ¹⁾

Eckhardt, Michael ^{1)*}

Goulson, David ^{2)*}

Heinzmann, Daniel ^{1)*}

Lange, Carlos ^{3)*}

Plischuk, Santiago ^{3)*}

Ruz Escudero, Luisa ^{4)*}

Salathé, Rahel ^{1) 5)*}

Scriven, Jessica J. ^{2)*}

Schmid-Hempel, Paul ¹⁾⁺

*) in alphabetical order

¹⁾ ETH Zürich, Institute of Integrative Biology (IBZ), ETH-Zentrum CHN, Universitätsstrasse 16,
CH-8092 Zürich, Switzerland

²⁾ University of Stirling, Biological and Environmental Sciences, School of Natural Sciences,
Stirling, Scotland FK9 4LA

³⁾ Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CCT La Plata CONICET-UNLP, and
Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CICPBA), Calle 2 #

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584 (1900) La Plata, Argentina

4) Pontificia Universita Católica de Valparaíso, Instituto de Biología, Avda. Brasil, 2950
Valparaíso, Chile

5) present address: Penn State University, Dept. of Biology, University Park, PA 16801, USA

*) corresponding author: Fax +41 44 632 1217, email: psh@env.ethz.ch

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Summary

1. The palaeartic *Bombus ruderatus* (in 1982/83) and *B. terrestris* (1998) have both been introduced into South America (Chile) for pollination purposes. We here report on the results of sampling campaigns in 2004, and 2010-2012 showing that both species have established and massively expanded their range.
2. *B. terrestris*, in particular, has spread by some 200 km /year and had reached the Atlantic coast in Argentina by the end of 2011. Both species, and especially *B. terrestris*, are infected by protozoan parasites that seem to spread along with the imported hosts and spill over to native species.
3. Genetic analyses by polymorphic microsatellite loci suggest that the host population of *B. terrestris* is genetically diverse, as expected from a large invading founder population, and structured through isolation by distance. Genetically, the populations of the trypanosomatid parasite, *Crithidia bombi*, sampled in 2004 are less diverse and distinct from the ones sampled later. Current *C. bombi* populations are highly heterozygous, and

also structured through isolation by distance correlating with the genetic distances of *B. terrestris*, suggesting the latter's expansion to be a main structuring factor for the parasite.

4. Remarkably, wherever *B. terrestris* spreads, the native *B. dahlbomii* disappears although the reasons remain unclear. Our ecological and genetic data suggest a major invasion event that is currently unfolding in southern South America with disastrous consequences for the native bumblebee species.

Introduction

Today, the invasion of species into foreign regions, that is, the spread of a species in an area where it was previously not found, and the resulting mixing of the world's biota are on the rise, being greatly facilitated by human travel and international trade (PYŠEK *et al.* 2010). In addition, animals or plants are purposefully imported into a new area by man because of their anticipated beneficial effects for agricultural produce or to control pests. Such biological invasions can become detrimental when the imported species escapes control and subsequently causes economic losses to crops or livestock (VILA *et al.* 2010) or starts threatening the native fauna and flora. However, whether or not an invasion is successful is assumed to depend on various factors, such as resource supply (PYŠEK *et al.* 2010), introduction effort and founder diversity (LOCKWOOD, CASSEY & BLACKBURN 2005), or the invader's affiliation with human activities (JESCHKE & STRAYER 2006). A more recent emphasis is on the effects of parasites, e.g. when resident parasites select against invaders, novel parasites select against

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residents, or when invaders leave behind their parasite fauna ("enemy release hypothesis") (CROWL *et al.* 2008; PRENTER *et al.* 2004).

Cases of purposeful introductions of foreign animals and plants are often well documented and the agenda associated with the introduction is clear. Such cases can therefore elucidate patterns and, sometimes, factors that mediate the invasion process. Here, we report on one such case - the planned introduction of European bumblebees for pollination services into southern South America. Bumblebees are important pollinators in their natural range (BINGHAM & ORTHNER 1998). As commercially produced pollinators they add substantially to the economic value of agricultural products (GOULSON 2003; VELTHUIS & VAN DOORN 2006). The commercial trade is mainly in two species, *Bombus terrestris* L. in Europe and *B. impatiens* Cresson (*B. occidentalis* Greene to a lesser extent) in North America, both of which are bred in the hundreds of thousands of colonies mainly for greenhouse pollination of valuable crops (VELTHUIS & VAN DOORN 2006). At the same time, the natural abundance and diversity of bumblebee species is in decline in many parts of the world (BIESMEIJER *et al.* 2006; GOULSON, LYE & DARVILL 2008; WILLIAMS 1982), with parasites being identified as drivers of these declines (CAMERON *et al.* 2011). There is therefore growing concern about pathogen transport with imports and spill-over to resident pollinator communities due to the fact that non-native, commercially raised bumblebees are being exported and have now established in various regions of the world (MEEUS *et al.* 2011). These colonies commonly contain parasites (MURRAY *et al.* 2013). Cases in point are the import and subsequent naturalization of *B. terrestris* into Northern Japan (Hokkaido) in the 1990s (INOUE, YOKOYAMA & WASHITANI 2008) with concerns of

parasite spill-over (GOKA, OKABE & YONEDA 2006; NIWA *et al.* 2004). Several *Bombus* species from England were introduced and established in New Zealand and have brought parasites with them (HOPKINS 1914; MACFARLANE & GRIFFIN 1990).

Here, we report on the case of two European *Bombus* having been introduced for pollination in southern South America (Chile) in the 1980s and 1990s. We aimed at analysing the historical spread of these species, their population genetic structure and the presence of two common parasites, *Crithidia* (Trypanosomatidae) and *Nosema* (*Paranosema*, Microsporidia) to scrutinize the extent of the invasion and to elucidate the possible role of parasitic infections in this process. In fact, concerns over parasitism as a cause for the displacement of native species have recently resurfaced (AIZEN, LOZADA & MORALES 2011). However, prior to our first campaign (2004) next to nothing was known about the parasites and diseases of *Bombus* in southern South America, but reports have multiplied since (ARBETMAN *et al.* 2013; MAGGI, LUCIA & ABRAHAMOVICH 2011; PLISCHUK & LANGE 2009b; PLISCHUK *et al.* 2009; PLISCHUK *et al.* 2011a). Yet, this report represents the first systematic survey to date.

Introduction of *Bombus* spp. into South America

Of the 250 species of bumblebees (*Bombus* spp.) (CAMERON, HINES & WILLIAMS 2007), a total of 24 species have naturally reached South America (CAMERON & WILLIAMS 2003; HINES 2008). Here, we focus on the Patagonian region (the south of Argentina and Chile) that harbours five native species. We did not encounter *B. atratus* Franklin, *B. bellicosus* Smith, or *B. funebris* Smith in this study, either because their known distributional ranges do not, or only marginally

overlap, with our sampling locations (ABRAHAMOVICH & DÍAZ 1982; ABRAHAMOVICH, DIAZ & LUCIA 2007; ABRAHAMOVICH, DIAZ & MORRONE 2004) but we did find *B. dahlbomii* Guérin-Méneville and *B. opifex* Smith.

INTRODUCTION OF *B. RUDERATUS*

For importation, European *B. ruderatus* Fabricius were collected in New Zealand (where it had previously been introduced in 1882) in December 1982 (199 queens) and November 1983 (192 queens), and shipped to Chile (ARRETZ & MACFARLANE 1986). New Zealand was chosen due to the synchronicity in the breeding cycle with South America and the assumed low abundance of parasites at sampling locations. The sampling sites were believed to be free of the nematode, *Sphaerularia bombi* Dufour 1837, and the tracheal mite, *Locustacarus buchneri* Stammer 1951. They were also assumed to be free of the protozoan parasite *Apicystis (Mattesia) bombi* Lipa & Triggiani 1996, as this gregarine is reported to be missing in New Zealand (see MACFARLANE, LIPA & LIU 1995, for a review). Several other mite species in New Zealand were also known from the native South American *B. dahlbomii* (ARRETZ & MACFARLANE 1986) and thus not considered a problem. Queens of the first shipment were released on 23 December 1982 east of the city of Temuco (100 queens released near Coipue; 45 released near Cunco) in an area of red clover production. One hundred queens of the second shipment were released on 6 December 1983 near Coipue, and an additional 69 queens on 7 December 1983 near Cunco (Figure 1) (ARRETZ & MACFARLANE 1986). Introductions were successful because workers of *B. ruderatus* were seen near Coipue in February and March 1983 and in the following southern spring (November/December 1983) about 7 km south of Cunco.

INTRODUCTION OF *B. TERRESTRIS*

The information on the release of *B. terrestris* in Chile is much less detailed (RUZ 2002; L. Ruz, pers. comm). One source was from Israel, likely from the Mt. Carmel region, where *B. terrestris* became the dominant species (DAFNI & SHMIDA 1996) after having been first recorded in the early 1930s in the North of Israel. A possible second source was from Belgium but details of this shipment and its release are missing. Imports were authorized by the Servicio Agrícola y Ganadero (SAG, Chile) in 1998 (RUZ 2002). A company (Xilema S.A.) handled the actual introductions from Israel (see reports by the FIA, Fundación para la Innovación Agraria; FIA-GOBIERNO DE CHILE 2000). Xilema also ran tests, on behalf of the FIA, for pollination efficiency on tomato in the experimental station of the Pontificia Universidad Católica de Valparaíso 's School of Agriculture near Quillota (*c.f.* Figure 1) during 1997 /1998 using both closed and open glasshouses (FIA-GOBIERNO DE CHILE 2000; project code FIA-PI-C-1997-2-A-009). According to FIA, Xilema had continued to import bumblebees after the termination of the project (i.e. after 1998); further companies are said to have applied to SAG for import permissions but no further information is available. The FIA had furthermore run a project to breed *B. terrestris* in the years 2008-2009 (project code 08CS-1111). According to Prof. Eugenio Lopez (School of Agronomy, Valparaíso University; contacted in early 2012; L. Ruz, pers.comm.) the first shipment from Israel in 1997 was used within the FIA-project in Quillota; the second shipment from Israel in 1998 was distributed to farmers across Chile in Arica, Copiapó, San Felipe, Quillota, Limache, Santiago, and Los Angeles (MONTALVA *et al.* 2011). Given this historical information, we tentatively set the introduction point of *B. terrestris* relevant for this study to the year 1998 in Quillota. In addition to the two European species, the North American *B.*

impatiens was also imported by farmers but no further details are known (Ruz 2002). No specimens of this species were encountered during our studies; hence it probably did not establish.

Material and methods

SAMPLING

We did several explicit sampling campaigns (Figure 1). *Campaign (1)*: In January 2004, one of us (RS) sampled the area around Villarrica (Chile). *Campaign (2)*: In January - February 2010, sampling was done by PSH and RSH in Chile from ca. 100 km north of Copiapó (administrative Region III) to the island of Chiloé (Region X). *Campaign (3)*: In January 2011, PSH and RSH surveyed Patagonia (along Ruta Nacional, R.N. 40) from San Carlos de Bariloche, Argentina, to Punta Arenas (Chile) and along the Atlantic coast (R.N. 3 to Comodoro Rivadavia). *Campaign (4)*: in October - November 2011, SP checked the area around Comodoro Rivadavia for *B. terrestris*. *Campaign (5)*: In January 2012, DG and JS surveyed the eastern slopes of the Argentina Andes, from Bariloche north towards Mendoza. For details see Table S1, Supporting Information. Generally, we aimed at around 50 specimens per site, but this could not always be achieved due to weather and local conditions.

Sampling locations were grouped into larger geographical regions: (1) "Chile Central" (sampled in 2004: "Chile Central 2004") representing the core Chilean mainland from Santiago south to Puerto Montt; (2) "Andes East" - the eastern slopes of the Andes, from San Martín de los Andes (where *B. terrestris* was first reported in Argentina) south to Gobernador Costa; (3) "Argentina

Atlantic": the reach from inland, Sarmiento, to the Atlantic coast at Comodoro Rivadavia; (4) "Patagonia West": the Patagonian region on the western slope of the Andes; and (5) "Patagonia South": the southern tip of South America, especially the area from around Puerto Natales to Punta Arenas. The sampling periods (January-March, except campaign 4 to the Atlantic coast) coincided with the known phenology of *Bombus* in these areas, e.g. ABRAHAMOVICH & DIAZ 2001; AIZEN 2001.

During the sampling campaigns, sites along the major roads with abundant flowers typically visited by bumblebees were searched. These sites consisted of flowering patches either directly along the road or land that could easily be reached by foot in the vicinity. The decisions were made on the spot, such as to sample native bumblebee flowers known to be visited by the native *Bombus* species. Examples include amancay (*Alstroemeria aurea*), almost exclusively having been visited by the native *B. dahlbomii* (AIZEN 2001) especially in forested or bushy areas, *Fuchsia (magellanica)*, e.g. abundant in many places along Lago General Carrera, various Fabaceae (e.g. *Adesmia*), other flowering plants (e.g. *Phacelia*, Boringiaceae), but also more bush-like vegetation (*Luma* spp.). In addition, imported plants such as clover (*Trifolium*) and Asteraceae (*Carduus*) were also prominent at many places. Note that *B. dahlbomii* was also found in large numbers along our "road sites" in the very same vegetation as *B. terrestris*, such that the differences reported here are not simply due to a possible sampling bias.

Sites were separated by at least 10 - 20 km, but generally separated by larger distances (coordinates, see Table S1, Supporting Information). Bees were collected at random by walking slowly through a site and captured when visiting flowers, either by directly pushing them into

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sampling vials or by netting as appropriate. The individuals were put into highly concentrated alcohol (> 90 % ethanol) either immediately after sampling, or first individually stored in a cooler and put into alcohol in the evenings of every sampling day. Species and sex/caste was identified at capture but verified in the laboratory later. However, all species sampled here (*B. terrestris*, *B. ruderatus*, *B. dahlbomii*, and *B. opifex*) are readily distinguishable from one another by visual inspection alone (ABRAHAMOVICH, DIAZ & LUCIA 2007).

DNA EXTRACTION AND HOST GENOTYPING

Only genetic data from *B. terrestris* females are reported here due to their abundance (sample sizes), wide distribution, and the availability of many polymorphic microsatellite loci. Genomic DNA (also used for parasite identification, see below) was extracted after dissection from whole guts using the DNeasy 96 Blood & Tissue Kit[®] (Qiagen GmbH, Hilden, Germany) following the instructions by the manufacturer. Note that no *B. terrestris* were present in the 2004 samples. For *B. terrestris*, we used seven microsatellites developed by (ESTOUP *et al.* 1993) but adapted the protocol as follows. We ran two multiplex PCR reactions: "BB-Msat1" combined the PCR-reaction for primers B100, B118, B124, B126 and B132 (annealing temperature = 57° C); "BB-Msat2" combined the reaction for primers B10 and B11 (annealing temperature = 52°). BB-Msat1 and BB-Msat2 were mixed before loading the reactions onto an ABI 3730 sequencer (Applied Biosystems). Fragments were further analysed with the software Peak Scanner[™] (Applied Biosystems), and all results inspected visually and checked for consistency and clarity of signal.

PARASITE IDENTIFICATION AND GENOTYPING

Infections by the gut parasite, *Crithidia* (Trypanosomatidae) were genotyped from all host species. We checked for infection status (yes/no) with the Cyt b sequence (SCHMID-HEMPEL & TOGNAZZO 2010), and infection status for *Nosema bombi* Fantham & Porter 1914 (Microsporidia) with the small subunit rRNA (using the primers 18f and 1537r from BAKER *et al.* 1995). For this, PCR products from all bees were visualized on PCR CheckIT® gels (Elchrom Scientific, Baar, Switzerland) together with the necessary controls. Presence of a band was taken as positive for infection, absence as negative. PCR-products from positive infections were purified (ExoSAP method; HANKE & WINK 1994; WERLE *et al.* 1994), and directly sequenced using BigDye® chemistry on a 3130/3730 ABI Sequencer (Applied Biosystems). Sequences were edited and blasted for parasite identification using the software MacVector 12.5.1 (Mac Vector Inc., Cary, NC). For *Crithidia*, all positives were of Cyt b-type "A1", defining them as species *C. bombi* Lipa & Triggiani 1988 (SCHMID-HEMPEL & TOGNAZZO 2010). Therefore, a subsequent PCR was performed for the five *C. bombi* microsatellites loci Cri4G9, Cri4, Cri16, Cri2.F10, and Cri1B6 (REBER FUNK, SCHMID-HEMPEL & SCHMID-HEMPEL 2006) to define the genotype. Two multiplex PCR (CB-Msat1 and CB-Msat2) reactions were done as described in (SCHMID-HEMPEL *et al.* 2011). The two multiplex reactions were genotyped separately on an ABI 3730 (Applied Biosystems). Fragments were analysed using the software Peak Scanner™ (Applied Biosystems) and again visually inspected. DNA samples from 2004 had been extracted during an earlier study (SALATHÉ ZEHNDER 2007) using 10 % Chelex®100 (BioRad), mixed with an equal volume of Ringer solution (Merck). At the time, the samples were analysed for infection status (yes/no) by one of us (RS); these infection status data were used here. For the

current combined study, these extractions were retrieved from storage but, unfortunately, could no longer be used for a re-analysis. Therefore, we had to use a sub-sample of the infected bees from 2004 for which the guts had been stored at -20 °C (hence, not all infected bees of 2004 could be fully genotyped).

STATISTICAL ANALYSES OF GENETIC DATA

To analyse the genetic data we used GENEPOP (RAYMOND & ROUSSET 1995), R-packages pegas (PARADIS 2010), adegenet (JOMBART 2008), hierfstat (GOUDET 2005), and the program STRUCTURE (PRITCHARD, STEPHENS & DONNELLY 2000); for geographical rendering: R package maptool (LEWIN-KOH *et al.* 2009). For the genetic analyses of *B. terrestris*, populations with less than five individuals were merged with a neighbouring one whilst respecting geographic barriers (e.g. not across the Andes; *c.f.* Table 2). This left us with a total of 28 populations. Re-running the major analyses by simply excluding all small populations did not change any of the conclusions. Because not all individuals were infected, sample sizes for the *Crithidia* data are smaller. We again merged small populations into neighbouring larger ones in a similar vein as above. Given the generally smaller samples, we required at least three individuals per population for genetic analyses. (*c.f.* Table S2, Supporting Information). In a number of cases, the genotyping of infections showed more than two alleles at one or several loci. Since *C. bombi* is strictly diploid, this indicates a multiple genotype infection. We have used the method used in (SALATHÉ & SCHMID-HEMPEL 2011) to resolve multiple genotype infections into the single genotypes co-infecting a host, which was possible in all cases. We have then used these resolved single genotypes for the analyses reported here. An alternative method (Majority

Rule) used the most frequent genotypes as defined by allele frequencies in region. The corresponding details of method and the results are given in the Supplementary Information for comparison. The results from both methods were qualitatively the same.

Results

THE DISTRIBUTION AND SPREAD OF THE IMPORTED SPECIES

The original distribution of the native species, especially *B. dahlbohmi*, and prior to the 1990's is summarized in (MONTALVA *et al.* 2011) and shown in Figure 1. Furthermore, *B. ruderatus* had crossed the Andes and was first reported from the Río Negro province, Argentina, in 1996 (ROIG ALSINA & AIZEN 1996; cited in ABRAHAMOVICH, TELLERIA & DIAZ 2001). Our first campaign of 2004 was restricted to the Chilean Lake district (region IX, around Villarrica and Pucón); a total of 291 bees could be collected. Only two species were found (*B. dahlbohmi*, *B. ruderatus*). In the subsequent campaigns of 2010 and later, *B. terrestris* was found over a wide area (Figure 2, Table 1). In particular, *B. dahlbohmi* common in 2004 in the Lake district had in the meantime disappeared from this area. During the 2010 campaign the area from Santiago north to Copiapó was also visited (during January 2010), but no *Bombus* specimen could be found there.

In the campaign of 2011, *B. terrestris* was found on the Chilean side of the Andes as far south as Villa O'Higgins (Aysén region; Figure 2). In January/February 2011, the invasion front of *B. terrestris* was likely spreading along the river Baker and the shores of Lake General Carrera, as suggested by the many sexuals found there (see Table S1 Supporting Information; males in *B. terrestris*, young queens in *B. dahlbohmi*). Moreover, whereas only *B. dahlbohmi* were found at

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one sampling point along Baker (site Río Baker I), only *B. terrestris* were found 10 - 20 km further downstream (Río Baker II), thus forming a relatively sharp boundary in an apparently homogeneous habitat. *B. terrestris* seemed to be spreading eastward along the northern shore of lake General Carrera and had just reached Los Antiguos (Argentina) at its eastern end. Along the northern lake shore only *B. terrestris* were found (sites Lago Gral Carrera I, II; 70 - 90 % being males); and only *B. dahlbomii* along the southern shore (connecting to the Río Baker valley; sites Lago Gral Carrera III, IV). Sampling in a corridor crossing the Patagonian steppe suggested that the spread of *B. terrestris* in early 2011 had reached a point between Sarmiento and the Atlantic port of Comodoro Rivadavia. No specimens of the invader were found at this time on the coast itself despite intensive searching. Yet, sampling in October-November 2011 showed that *B. terrestris* was now present in large numbers around Comodoro Rivadavia (Table 1, Figure 2). In the campaign of 2012, *B. terrestris* was found on the eastern slopes of the Andes at a considerable distance north of its first sighting near San Martín de los Andes (Neuquén province) in 2006 (TORRETTA, MEDAN & ARAHAMOVICH 2006) (Table 1, Figure 2). The native species *B. opifex* was only met around San Rafael where *B. terrestris* was not present at that time.

Our campaigns of 2004 and 2010 confirmed the distributional status of *B. ruderatus* as described by MONTALVA *et al.* 2011; specimens were collected in an area reaching from Temuco to Puerto Montt, Chile. In 2011 we collected *B. ruderatus* in small numbers also on the eastern slopes of the Andes, from Bariloche south to Corcovado (Table 1, Figure 2).

PARASITE INFECTIONS

Crithidia bombi. Among all species, the average *C. bombi* infection prevalence was 14.3 %, with a peak value of 80 % for population Los Angeles, and substantial infections in Central Chile and the region of Puerto Montt - Bariloche (Figure 3; Table S2 Supplementary Information). A total of 183 hosts infected by *C. bombi* could be fully genotyped with microsatellites. Of those, 41.5 % contained multiple infections; this proportion was not different among host species (*B. dahlbomii*: 44 %, *B. ruderatus*: 36 %, *B. terrestris* 41 %; Table S3, Supporting Information).

Host species differed in infection prevalence ($X^2 = 17.22$, $P < 0.001$, $df = 3$, $n = 1'620$) with 18.5 % for *B. dahlbomii* ($n = 378$), 7.7. % for *B. ruderatus* ($n = 196$), 14.4 % for *B. terrestris* ($n = 1'018$), and none in *B. opifex* ($n = 28$). Thus, 70 of the total of 232 infections (30.2%) were carried by the native species *B. dahlbomii*. Significant differences were also found among populations ($X^2 = 351.04$, $df = 49$, $P < 0.0001$; $n = 50$ populations) and regions ($X^2 = 93.77$, $df = 5$, $P < 0.0001$; $n = 6$ regions; see Table S2 Supplementary Information for additional GLM-analyses), but these effects are partly confounded with species.

Nosema bombi. Microsporidian infections were identified by rRNA sequences. The closest matches were to *N. bombi* that was prevalent in the Chile samples, matches to *N. thomsoni* and *N. portugal* were mainly found in Argentina, and a few matches to *Vairimorpha* sp. at several locations. Overall, 2 % of the host bees carried a microsporidian infection ($n = 1,295$ bees; Table S2, Supporting Information); none was found in *B. dahlbomii* ($n = 198$) or *B. opifex* ($n = 28$), but 6.5 % ($n = 77$) in *B. ruderatus*, and 2.0 % ($n = 992$) in *B. terrestris* (among species $X^2 = 12.95$, df

=3, $P = 0.005$). All *Nosema* infections were thus contained within the imported species.

Although *N. bombi* was too rare for reliable analyses, nominally, populations and regions showed significant differences (Table S2, Supporting Information). Region "Patagonia West" (prevalence 10.9 %, $n = 128$ hosts) stood out due to high infection levels in *B. terrestris* that were sampled along lake General Carrera. In all, prevalences of *C. bombi* and *N. bombi* were not associated ($X^2 = 0.034$, $df = 1$, $P = 0.85$; for population means: Spearman's $r = 0.249$, $n = 37$, $P > 0.1$).

GENETIC POPULATION STRUCTURE OF *B. TERRESTRIS*

A total of 818 individuals could be successfully genotyped out of 1,018 collected female *B. terrestris*, representing 28 populations with at least 5 individuals (Table 2). Some specimen could not be typed because of technical non-amplification (not null alleles); in some cases, locus B118 did not amplify. All of the seven loci were polymorphic and, in any population, had an average of around 5 alleles, with a conspicuously low value (3.2 ± 0.77 alleles) for the Atlantic coast population of Comodoro Rivadavia. Similarly, observed heterozygosity value, H_{obs} , and genic diversity, H_s , were relatively high (Table 2, Table S4 Supporting Information). Almost all loci, in all populations, were in HW-equilibrium; only 11 out of the 231 tested values (4.8 %) were significantly deviating at the $P < 0.0001$ level (Bonferroni-corrected threshold), presumably chance significances. Thus, *B. terrestris* populations in South America are genetically variable and nominally in HW-equilibrium.

The populations of *B. terrestris* show some differentiation among one another ($G_{st} = 1,233.85$, $X^2 = 1,528.6$, $P < 0.001$) (GOUDET 2005; GOUDET *et al.* 1996). Analysis by the program STRUCTURE (PRITCHARD, STEPHENS & DONNELLY 2000) using the method of EVANNO, REGNAUT & GOUDET (2005) generated $K = 3$ sub-populations, primarily with bees from the Atlantic coast (Comodore Rivadavia) and, to some degree, from central Chile being separated from the remaining ones (App S1, Supplementary Information). No structuring was visible when using the ratio of pairwise $F_{st}/(1-F_{st})$ as suggested by FRANCOIS *et al.* (2010) for a Principal Component Analysis of genetic distance (JOMBART, DEVILLARD & BALLOUX 2010) (Figure S1, Supplementary Information). Similarly, Genetical Correspondance Analysis (JOMBART, DEVILLARD & BALLOUX 2010), and the Monmonier algorithm (R package hierfstat) also failed to identify clear boundaries between populations. A nearest-neighbour joining tree suggested that the geographically far spread populations were indeed also genetically far away (Figure S2, Supplementary Information). Instead of separated populations, differences among the 28 populations seem to result from an effect of isolation-by-distance (Fig. 4a). We also checked for a relationship of genetic distance, the $F_{st}/(1-F_{st})$ ratio, with the physical distance from the likely source of introduction (here: population Docas). But neither populations of *B. terrestris* (Spearman's $r = 0.04$, $P = 0.83$, $n = 25$ populations) nor those of *C. bombi* (Spearman's $r = -0.32$, $P = 0.13$, $n = 23$ populations) showed any such correlation.

GENETIC POPULATION STRUCTURE OF *C. BOMBI*

The population genetic structure of *C. bombi* infections collected in the 2004 campaign showed lower genetic variation as compared to infections from the later campaigns (Table 3). For

example, allelic richness for 2004 (average 1.95 ± 1.00 S.D. alleles, $n = 5$ populations, rarefaction minimum size) was lower than in the populations sampled later (campaigns 2010-2012: 2.63 ± 0.81 S.D., $n = 19$; Man-Whitney $U = 435$, $p = 0.003$): differences were visible even in the same location (e.g. the Pucón sample in 2004 vs. 2010, Table 3). An exception was locus Cri16 that was completely monomorphic with only one allele (size 119) throughout all samples (Table S5, S6 Supplementary Information). All further genetic analyses were therefore run with locus Cri16 excluded. Apart from two out of 70 tests, all loci and populations were in HW-equilibrium (at a Bonferroni-corrected value of $P < 0.0001$); three significant values likely are chance positives.

Overall, the population of *C. bombi* in the area is structured ($G_{st} = 60.64$ ($X^2 = 73.1$, $P < 0.001$), and an analysis by the program STRUCTURE resulted in three populations. However, only the population of the 2004 campaign was clearly different from the remaining ones (see Appendix S1, Supplementary Information). Therefore, rather than having clearly distinct sup-populations, isolation-by-distance seems the most defining feature of *C. bombi* populations; this is true for the later campaigns (see Figure 4b) but also when tested for all samples (Spearman's $r = 0.138$, $P = 0.022$, $n = 276$; $m = 24$ populations). A neighbour-joining tree suggested that the samples collected in 2004 are set apart from those collected later and that infections from the eastern slopes of the Andes were close to those from southern Central Chile (Figure 5). In explicit tests, *C. bombi* infections were genetically differentiated between the 2004 campaign (all hosts, $n = 39$ infections) and later (2010-2012) campaign, but not different among host species; *B.*

terrestris ($n = 134$), *B. dahlbomii* ($n = 3$), or *B. ruderatus* ($n = 4$). A G-test also separated early and late (in GENEPOP) at every locus and overall ($P < 0.0001$ in all cases).

Finally, we looked at the association of hosts and parasite genetic distances, which was possible for a subset of $n = 106$ infected host individuals from 22 populations. No evidence for any association between genotypes *per se* was found - as is to be expected for neutral markers. However, the pairwise genetic distances of hosts or parasites from the same populations correlated rather well (Figure S3, Supplementary Information).

Discussion

The introduction of two palaearctic bumblebee species into South America - *B. ruderatus* in 1982/83 and *B. terrestris* in 1998 - has led to a dramatic invasion process as both are now found far from their introduction sites. *B. ruderatus* is now well established in mainland Chile as well as on the eastern slopes of the Andes (Table 1, Figure 2). It crossed into Argentina around the Bariloche region in 1994 (ROIG ALSINA & AIZEN 1996) and coexisted with the native species until *B. terrestris* arrived there in 2006 (TORRETTA, MEDAN & ARAHAMOVICH 2006). The situation for *B. terrestris* is much more dramatic. By the beginning of 2010, it was by far the dominant species in the agricultural mainland of Chile, from Santiago to Puerto Montt and, possibly, Chiloé Island (where we spotted a few *B. dahlbomii* and *B. terrestris* in the 2010 campaign). By 2011/12, it had spread in Patagonia south to a point near Villa O'Higgins in Chile, and had reached the northern and eastern shores of Lago General Carrera (named Lago Buenos Aires in Argentina). Furthermore, by February 2011 - despite intensive monitoring - no *B.*

terrestris was found along the R.N. 26 between Comodoro Rivadavia and Sarmiento in Argentina. Yet, by October 2011 it had reached the Atlantic coast (Figure 2). *B. terrestris* was first seen east of the Andes in early 2006 in the region of San Martín de los Andes (TORRETTA, MEDAN & ARAHAMOVICH 2006), presumably having crossed the mountains there as the passes to the north, reaching over 4,000 m, are presumably impossible to cross. By 2012, *B. terrestris* had spread further north and south along the eastern slopes of the Andes (Figure 2).

The fate of the native *B. dahlbomii* is especially remarkable as our distributional data suggest a rapid displacement by *B. terrestris*. A few examples illustrate this. In 2004, the native *B. dahlbomii* and the introduced *B. ruderatus* were abundant around the Chilean Lake district of Villarrica - Pucón (Table 1, Figure 2). But by 2010, *B. terrestris* had become the dominant species whereas *B. dahlbomii* was no longer found. During the 2010/2011 campaigns we noticed clear boundaries between the advancing *B. terrestris* and the presence of the native *B. dahlbomii* in Southern Patagonia and around Lake General Carrera. Furthermore, *B. ruderatus* was present around Bariloche and San Martín de los Andes, but had become much less abundant after *B. terrestris* had arrived (TORRETTA, MEDAN & ARAHAMOVICH 2006). It seems therefore that wherever *B. terrestris* appears, the native *B. dahlbomii* disappears whilst, interestingly, *B. ruderatus* manages to persist at low abundance. Most spectacularly, the advance of *B. terrestris* has been in the order of 200 km per year - an astonishing figure even given the vast spatial scale of Patagonia. Since not only sexuals but also workers are found in all locations (Table S1, Supplementary information), viable colonies must have established in the

advancing front as workers are assumed not to venture farther than 1 - 2 km from their nest (DARVILL, KNIGHT & GOULSON 2004; KNIGHT *et al.* 2005; OSBORNE *et al.* 1999).

On the western (Chilean) side of the Andes studied here, the habitats are generally temperate in climate, covered with vegetation, forests, and agricultural lands. Hence, *B. terrestris* will find suitable conditions there and had established in most of this area around 2008 at the latest (Figure 2). Along the eastern slopes of the Andes, *B. terrestris* is found where similar - albeit generally drier than in the West - habitat conditions prevail. By contrast, virtually all of Patagonia between the foothills of the Andes and the Atlantic coast is dry steppe with scarce floral resources. Given where we found the bees during the 2011 campaign, *B. terrestris* seems to spread across the steppe along the major river systems draining towards the Atlantic. These river systems offer moist conditions and more abundant floral resources. The prevailing (and sometimes very strong) westerly winds across the Andes no doubt aid this dispersal. Note that in its European native range, *B. terrestris* does not extend into the far North (this is, only up to ca. 60° N in Scandinavia) but is widespread throughout the Mediterranean, a region with dry summers and abundant floral resources in a moist winter. Furthermore, our genetic analyses suggest that virtually all populations of *B. terrestris* are as genetically diverse as the core populations of mainland Europe (ESTOUP *et al.* 1996; SCHMID-HEMPEL *et al.* 2007) (Table 2). As of 2012, the overall population of *B. terrestris* is not separated into distinct sub-populations but shows isolation by distance (Figure 4).

Based on our data, we can therefore sketch a likely expansion scenario for *B. terrestris* (Figure 6). After an initial expansion across central Chile, which it dominated less than ten years after its release, multiple crossings across the Andes are likely to have occurred. Likely, a first

crossing around 2006 in the region of San Martín de los Andes, followed by others in the South, notably around L Gral Carrera. At the same time, *B. terrestris* spread farther south along the western side of the Andes, and later north and south along the eastern side. Finally, *B. terrestris* now rapidly expands east across the Patagonian steppe. Given the current speed of dispersal, it will probably reach the Strait of Magellan in a few years, perhaps only slowed down by the subantarctic climate of Southern Patagonia, as one might conclude by its absence from subarctic Scandinavia.

Elucidating a possible role of parasites for the invasion process, we found that parasites, notably *C. bombi*, is present at high prevalence. Furthermore, populations of *C. bombi* infections are genetically similarly structured as its main host, *B. terrestris* and a historical spread is suggested because infections were considerably less diverse in 2004 than later (Table 3) and can also be separated on a phylogeographic tree (Figure 5). Also, *B. terrestris* is known to have carried the infection in 2009 samples from around Bariloche, Argentina, at high levels (21.6 %) (PLISCHUK & LANGE 2009a), whereas it was not present in four native species collected in several parts of Argentina a few years earlier. Based on Figure 5, perhaps, *C. bombi* infections could have been brought in first by *B. ruderatus*, and then enriched in diversity by the later introduction of *B. terrestris*, with which it now spreads rapidly across vast scales. Genetic distances among populations of *C. bombi* do indeed co-vary with the genetic distances of its now main host, *B. terrestris* (Figure S3 Supporting Information).

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The apparently quick and irreversible suppression of *B. dahlbomii* by the advancing *B. terrestris* is an enigma and has been commented upon earlier by other observers (ARBETMAN *et al.* 2013; MORALES 2007). What possible processes could account for this? Ecological competition between the native species and the invaders occurs (e.g. MADJIDIAN, MORALES & SMITH 2008; MORALES & AIZEN 2006 for *B. ruderatus*; MONTALVA *et al.* 2011 for *B. terrestris*). For example, *B. terrestris* could ecologically outcompete the native *B. dahlbomii* through its more generalized flower use (MONTALVA *et al.* 2011) or by being more adapted to fragmented habitats (AIZEN & FEINSINGER 2003). It remains unclear though whether ecological competition can account for such a rapid displacement, and, at least for *B. ruderatus*, this has been considered unlikely (AIZEN, LOZADA & MORALES 2011). Nevertheless, the role of ecological effects for the displacement of *B. dahlbomii* remains to be studied.

As outlined above, a factor for displacement could be novel, introduced parasites. Is this likely in this case? Experimental evidence is lacking and will be difficult to establish for such large scales. However, our data together with literature reports can sketch some possibilities. For example, we found that *N. bombi* only occurs in the introduced species. PLISCHUK *et al.* (2009) reported that *N. ceranae* Fries 1996 - an emerging parasite of honeybees in Europe (KLEE *et al.* 2007; PAXTON *et al.* 2007) - is present in native bumblebee species in Argentina (*B. atratus*, *B. morio*, *B. bellicosus*). So far, only the pathology of *N. bombi* is confirmed, but the generally very low prevalence of *Nosema* in our samples (Table S2 Supporting Information) should speak against a major role for this pathogen. It is conceivable that the low prevalence of *N. bombi* is due to non-sampling of heavily affected (and non-flying) bees. Note, however, we detected

Nosema by molecular markers, which identify the presence of the parasite long before any spores can be seen or any pathological effects are noticeable. No infections by the gregarine, *Apicystis (Mattesia) bombi* (which we have not investigated here), were found in native species in Argentina sampled between 2005 and 2009 outside Patagonia ($n = 441$, *B. atratus*, *B. morio*, *B. bellicosus*, *B. opifex*, *B. tucumanus*; (PLISCHUK & LANGE 2009a), nor in samples of *B. dahlbomii* ($n = 52$) and *B. ruderatus* ($n = 30$) (ARBETMAN *et al.* 2013) collected between 1994 and 2012 in the area around Bariloche and San Martín de los Andes but before *B. terrestris* arrived. After the arrival of *B. terrestris* (before 2009), the prevalence of *A. bombi* for *B. terrestris* in January 2009 was 3.6 % ($n = 111$ bees; *C. bombi*: 21.6 %) (PLISCHUK & LANGE 2009a). By 2010 in the same area 12.1 % ($n = 107$) of *B. terrestris* were infected (PLISCHUK *et al.* 2011b). By 2012 (ARBETMAN *et al.* 2013) prevalence had increased to 47 % in *B. terrestris* ($n = 30$), 56 % in *B. ruderatus* ($n = 9$); and 11 % in *B. dahlbomii* ($n = 9$). By 2009, the parasite also occurred in honeybees in the Bariloche region (10.1 %, $n = 138$ for 2009/10) whereas it appears to be absent in the Pampas region further northeast (PLISCHUK *et al.* 2011b). The pathology of *A. bombi* parasite is not well researched (e.g. LIU, MACFARLAND & PENGELLY 1974) but it is considered virulent (SCHMID-HEMPEL 1998).

We now show that *C. bomb* is very widespread and abundant wherever *B. terrestris* is found (Figure 3, Table S2 Supplementary Information). Furthermore, its population genetic structure seems to match that of *B. terrestris*. According to PLISCHUK & LANGE (2009a), *C. bombi* appears not to be present in native species outside Patagonia. The primary pathogenic effect of *C. bombi* is to sterilize founding queens (BROWN, SCHMID-HEMPEL & SCHMID-HEMPEL 2003 for *B. terrestris*),

which leads to failure during colony founding and severely compromised reproductive success. This effect might be stronger in a species like *B. dahlbomii* that likely have never encountered this parasite before (OTTERSTATTER & THOMSON 2008); yet, this remains to be tested. Whatever further studies will find, only a novel, rather abundant sufficiently virulent parasite should have those rapid effects that could indeed reduce the native population in a short matter of time. Both, *A. bombi* and *C. bombi* would qualify for this.

CONCLUSION

The introduction into South America of *B. ruderatus* and, especially of *B. terrestris*, for pollination purposes is having massive consequences. *B. terrestris* forms a healthy, genetically diverse population that is currently spreading at high rates and already has settled in a vast area, along with parasites, such as *Crithidia bombi*, *Noema bombi* and *Apicystis bombi*. In its wake, native *B. dahlbomii* populations diminished dramatically. Given its biology, *B. terrestris* has the potential to spread further north and south along the Andes and likely will reach Tierra del Fuego (where currently nothing is known about the native bumblebees) in a few years. Currently, *B. terrestris* spreads eastwards in Argentina along major river systems towards the Atlantic. *B. ruderatus* spreads much less aggressively and manages to co-exist with *B. terrestris* in places. The current changes in South America clearly are a stunning example of a rapid invasion event with potentially devastating consequences for the only remaining native *Bombus* species in Patagonia and a lesson for possible future introductions.

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References

Supporting Information

Appendix S1. Analysis of genetic data of *B. terrestris* and *C. bombi* with the program STRUCTURE.

Table S1. Synopsis of collection data.

Table S2. Synopsis of all infections per population.

Table S3. Single and multiple infections by *C. bombi*. **(a)** According to population. **(b)** According to host species.

Table S4. Genetic data per locus for *B. terrestris*.

Table S5. Genetic data per locus for *C. bombi*, based on resolved infections.

Table S6. Genetic data per locus for *C. bombi*, based on Majority Rule.

Figure S1. Principal Component Analysis for pairwise genetic distances among *B. terrestris* populations.

Figure S2. Neighbour-joining trees for genetic distances among *B. terrestris* populations. **(a)** Distances calculated as the ratio $F_{st}/(1-F_{st})$ after (FRANCOIS *et al.* 2010). **(b)** Distances calculated as Cavalli-Sforza distances (R-package hierfstat).

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Figure S3. Correlation of genetic distances among hosts, *B. terrestris*, and their *C. bombi* infections.

Figure legends

Figure 1. Original distribution of the native *B. dahlbomii* in southern South America, according to ABRAHAMOVICH, DIAZ & LUCIA (2007) and MONTALVA *et al.* (2011); this species occurs south and west of the dashed line except for the interior of the Patagonian steppe (are marked by the dotted line). *B. dahlbomii* is typically associated with more or less forested areas. Release sites of imported bumblebees in Chile are indicated by arrows. The current invasion by *B. terrestris* most likely started from the research facilities at Quillota (based on RUZ 2002, MONTALVA *et al.* 2011); for *B. ruderatus* three release sites were used - (Coipue), Malleco, and Cunco, based on ARRETZ & MACFARLANE (1986) (arrows). No records are available for Tierra del Fuego. Sampling sites for the four campaigns are indicated by their population id (see Table 1, and Table S1, Supplementary Information).

Figure 2. Presence of the native species, *B. dahlbomii*, and the two imported, *B. terrestris* and *B. ruderatus* in Chile. Data for *ruderatus* and *dahlbomii* are from the 2004 sampling campaign; pie size proportional to sample size (see Table 1). Approximate data for the range of *terrestris* in 1998/99 are taken from MONTALVA *et al.* (2011). Asterisks indicate release sites for the two successfully imported species (open symbol: *B. ruderatus*; closed symbol: *B. terrestris*).

Figure 3. Prevalence of *C. bombi* infections in all hosts for the 2004 and later campaigns; pie size proportional to sample size (see Table S2 Supporting Information). Infections in the 2004

campaign (Lake district, Chile) are in *B. ruderatus* and *B. dahlbomii* (no *B. terrestris* present at the time); infections of later campaigns in all species but mostly in *B. terrestris*.

Figure 4 Isolation by distance in specimens collected during the campaigns of 2012 - 2102 in Chile and Argentina. Geographical distance is given by angular degrees (1 degree approx. 111 km) along the shortest distance; genetic distance is given by the ratio of pairwise $F_{st}/(1-F_{st})$ - values for polymorphic loci ($k = 7$ in *B. terrestris*, $k = 5$ *C. bombi*). **(a)** Pairwise isolation by distance in *B. terrestris* (Spearman's $r = 0.609$, $P < 0.001$, $n = 799$; $m = 28$ populations). **(b)** Pairwise isolation by distance in *C. bombi* (Spearman's $r = 0.482$, $P < 0.001$, $n = 171$; $m = 19$ populations).

Figure 5. Neighbour-joining tree for pairwise genetic distances ($F_{st}/(1-F_{st})$) of *C. bombi* infections. Infections from the 2004 campaign (dashed rectangle) separate. No *B. terrestris* were sampled in this campaign. Location 'Docas' (rectangle) is close to the introduction site of *B. terrestris*. Locations on the eastern slope of the Andes (Argentina) are in italics. Location 'C Rivadavia' is on the Argentinian Atlantic coast (*c.f.* Figure 1).

Figure 6. The putative invasion of *B. terrestris* into South America. The species was imported into Chile (asterisk) in 1998/99 and escaped soon afterwards. The lines and yearly progress depict a likely scenario according to the results of the sampling campaigns reported here. Note that low passes of the Andes between Chile and Argentina can probably not be crossed north of approximately the Temuco-San Martín de los Andes line.

Table 1 Number of individuals sampled per host species and population.

id ¹⁾	Population, Region	Date	<i>B.dahlbom ii</i>	<i>B opifex</i>	<i>B ruderatus</i>	<i>B terrestris</i>	Sample N
	Chile Central 2004						
05	Aguas grandes	16.1.2004	85	0	38	0	123
02	Campo Anders	18.1.2004	50	0	18	0	68
06	Cunco	17.1.2004	15	0	19	0	34
07	Pucón I	10.1.2004	14	0	0	0	14
01	Valdivia	15.1.2004	13	0	39	0	52
	Chile Central						
09	Docas	7.1.2010	5	0	0	7	12
10	Farellones	17.1.2010	29	0	0	0	29
11	Los Angeles	21.1.2010	0	0	0	30	30
12	Mininco	21.1.2010	0	0	4	48	52
13	Nahal 1	23.1.2010	0	0	1	4	5
14	Los Sauces	23.1.2010	0	0	32	25	57
15	Pucón	26.1.2010	0	0	12	41	53
16	Puerto Montt	27.1.2010	1	0	0	48	49
17	Ensenada	1.2.2010	1	0	8	5	14
18	Octay	1.2.2010	0	0	6	21	27
19	Talhuaca	4.2.2010	1	0	12	44	57
20	Cobquecura	6.2.2010	4	0	0	18	22
	Andes East						
22	Bariloche	14.1.2011	0	0	1	2	3
23	El Manso	14.1.2011	0	0	1	61	62
24	El Rincón	14.1.2011	0	0	0	61	61
25	Trevelin I	16.1.2011	0	0	0	33	33
26	Trevelin II	17.1.2011	0	0	3	42	45
27	Corcovado	17.1.2011	0	0	2	62	64
43	Gobernador Costa	7.2.2011	0	0	0	27	27
44	Villavicencio	4.1.2012	0	10	0	0	10
45	Luján de Cuyo	5.1.2012	0	9	0	0	9
46	San Rafael	6.1.2012	0	9	0	0	9
47	Malargue	7.1.2012	0	0	0	7	7
48	Pehuenches	7.1.2012	0	0	0	42	42
49	Chos Malal	8.1.2012	0	0	0	25	25
50	Picunches	8.1.2012	0	0	0	1	1
51	Zapala	9.1.2012	0	0	0	29	29
52	Lacar	10.1.2012	0	0	0	68	68
53	S. Martín de los Andes	10.1.2012	0	0	0	52	52
	Argentina Atlantic						
21	Comodoro Rivadavia	4.11.2011	0	0	0	96	96
41	Sarmiento	7.2.2011	0	0	0	10	10
42	Rio Saghuer	7.2.2011	0	0	0	1	1
	Patagonia West						
28	Coyhaique	18.1.2011	0	0	0	1	1
29	LGral Carrera I	19.1.2011	9	0	0	45	54

30	LGral Carrera II	19.1.2011	0	0	0	21	21
31	Rio Baker I	19.1.2011	6	0	0	0	6
32	Rio Baker II	20.1.2011	0	0	0	27	27
33	LGral Carrera III	20.1.2011	3	0	0	0	3
34	LGral Carrera IV	20.1.2011	2	0	0	0	2
35	Los Antiguos	21.1.2011	0	0	0	14	14
Patagonia South							
36	El Chaltén	23.1.2011	1	0	0	0	1
37	El Calafate	26.1.2011	12	0	0	0	12
38	Puerta Natales	29.1.2011	63	0	0	0	63
39	Punta Arenas I	31.1.2011	7	0	0	0	7
40	Punta Arenas II	31.1.2011	57	0	0	0	57
Total			378	28	196	1,018	1,620

¹⁾ Population id (running number) used in the maps (*c.f.* Figure1).

Table 2 Genetic data for *B. terrestris* females for the 2010-2012 campaign. Only populations with ≥ 5 individuals included.

No *B. terrestris* were found at the sampling locations during the 2004 campaign or in region South Patagonia.

id ¹⁾	Population, Region ¹⁾	Sampled ²⁾	Type ³⁾	n Allels ⁴⁾ (S.D.)	Hobs ⁵⁾ (S.D.)	Hs ⁶⁾ (S.D.)
Chile Central (2010)						
09	Docas	7	6	5.27 (1.21)	0.762 (0.131)	0.798 (0.088)
17	Ensenada	5	5	5.43 (1.40)	0.828 (0.214)	0.818 (0.107)
14	Los Sauces	25	20	5.13 (0.58)	0.805 (0.078)	0.803 (0.058)
12	Mininco	48	45	5.38 (0.84)	0.785 (0.083)	0.822 (0.046)
11, 13	Nahal *	7	7	5.51 (1.45)	0.762 (0.189)	0.838 (0.066)
18	Octay	21	21	5.21 (0.85)	0.801 (0.095)	0.808 (0.053)
15	Pucón	41	41	5.33 (0.58)	0.831 (0.053)	0.823 (0.036)
16	Puerto Montt	48	31	4.99 (0.81)	0.743 (0.111)	0.799 (0.059)
20	Cobquecura	18	16	4.98	0.830	0.791

				(0.72)	(0.129)	(9.066)
19	Talhuaca	44	40	5.46 (0.84)	0.791 (0.101)	0.825 (0.053)
		264	232			
	Andes East					
49	Chos Malal	25	9	4.61 (0.82)	0.873 (0.149)	0.744 (0.108)
27	Corcovado	62	57	4.86 (0.89)	0.723 (0.103)	0.772 (0.093)
22, 23	El Manso *	63	54	5.47 (0.72)	0.803 (0.049)	0.833 (0.039)
24	El Rincón	61	58	5.10 (0.61)	0.836 (0.057)	0.805 (0.039)
43	Gobernador Costa	27	27	5.12 (0.87)	0.787 (0.109)	0.794 (0.086)
52	Lacar	68	33	5.78 (0.70)	0.801 (0.071)	0.853 (0.038)
47	Malargue	7	7	3.49 (0.77)	0.857 (0.184)	0.702 (0.092)
48	Pehuenches	42	41	5.23 (0.71)	0.815 (0.067)	0.817 (0.050)
53	S Martín Andes	52	47	5.34 (0.66)	0.814 (0.093)	0.829 (0.037)
25	Trevelin I	33	8	4.48 (1.06)	0.724 (0.142)	0.710 (0.145)
26	Trevelin II	42	42	4.72 (0.66)	0.735(0.12 3)	0.754 (0.075)
50, 51	Zapala *	30	17	5.15 (0.93)	0.781 (0.116)	0.814 (0.056)
		639	400			
	Argentina Atlantic					
21	Comodoro Rivadavia	96	96	3.20 (0.77)	0.615 (0.187)	0.595 (0.156)
41, 42	Sarmiento *	11	11	5.07 (0.97)	0.584 (0.165)	0.782 (0.096)
		107	107			
	Patagonia West					
29	LGral Carrera I	45	39	4.54 (0.52)	0.777 (0.072)	0.742 (0.077)
30	LGral Carrera II	21	14	6.10 (3.10)	0.687 (0.193) \$	0.728 (0.073) \$

35	Los Antiguos	14	9	4.07 (0.81)	0.816 (0.115)	0.722 (0.083)
31	Rio Baker II	27	13	5.53 (3.40)	0.731 (0.187) \$	0.786 (0.082) \$
		107	75			
	Total	1,117	818			

1) Population id as in map (Figure 1). Note that populations with an asterisks were merged to contain ≥ 5 individuals: "El Manso" with "Bariloche", "Sarmiento" with "Rio Saghuer", "Zapala" with "Picunches", "Nahal" with "Los Angeles"; population "Coyhaique" removed (only 1 individual). Other populations with no *B. terrestris* not listed.

2) Number of *B. terrestris* females sampled in population.

3) Number of *B. terrestris* females genotyped for this study.

4) Average allelic richness (S.D.) over all loci; rarefaction for minimum sample sizes.

5) *Hobs*: fraction of heterozygotes observed over all loci, according to {Nei, 1987 #9783}.

6) *Hs*: Genic diversity (expected heterozygosity).

\$) In these samples, Locus B118 missing (non-amplifying) in population.

Table 3 Genetic data for *C. bombi* Only populations with ≥ 3 infections included, based on multiple infections.

(for single infections, see Supplementary Material).

id ¹⁾	Population, Region ¹⁾	Sample d ²⁾	Type d ³⁾	Genotypes ⁴⁾	Richness ⁵⁾ (S.D.)	<i>Hobs</i> ⁶⁾ (S.D.)	<i>Hs</i> ⁷⁾ (S.D.)
	Chile Central 2004						
05	Aguas grandes	15	15	22	1.86 (1.00)	0.443 (0.520)	0.319 (0.368)
02	Campo Anders	6	6	10	1.67 (0.83)	0.500 (0.577)	0.283 (0.331)
06	Cunco	4	4	5	1.94 (1.30)	0.450 (0.526)	0.319 (0.384)
07	Pucón I	6	6	9	1.67 (0.82)	0.500 (0.577)	0.281 (0.329)
01	Valdivia	9	9	12	1.82 (0.67)	0.542 (0.529)	0.328 (0.283)

	Chile Central						
09	Docas	5	5	7	2.69 (1.13)	0.750 (0.500)	0.539 (0.359)
17	Ensenada	6	6	8	2.73 (0.93)	0.750 (0.319)	0.568 (0.218)
14	Los Sauces	3	3	4	2.49 (0.77)	0.812 (0.375)	0.521 (0.200)
11	Los Angeles	24	23	40	2.50 (0.57)	0.756 (0.311)	0.551 (0.177)
12	Mininco	8	3	5	2.71 (0.75)	0.700 (0.346)	0.562 (0.164)
13	Nahal 1	4	2	3	2.25 (0.50)	0.833 (0.333)	0.500 (0.136)
18	Octay	8	7	12	2.82 (1.04)	0.625 (0.369)	0.570 (0.292)
15	Pucón	3	3	3	2.75 (1.26)	0.750 (0.500)	0.542 (0.369)
16	Puerto Montt	10	10	13	2.61 (0.68)	0.827 (0.346)	0.574 (0.208)
20	Cobquecura	9	9	13	2.61 (0.57)	0.769 (0.294)	0.580 (0.174)
19	Talhuaca	4	4	4	2.30 (0.50)	0.750 (0.353)	0.489 (0.168)
	Andes East						
27	Corcovado	4	4	4	3.00 (1.08)	0.687 (0.314)	0.635 (0.269)
23	El Manso	17	16	20	2.83 (0.91)	0.750 (0.373)	0.597 (0.272)
24	El Rincón	26	25	37	2.87 (0.85)	0.824 (0.333)	0.615 (0.231)
48, 53	S Martín Andes)	3	3	4	3.01 (0.92)	0.875 (0.250)	0.646 (0.172)
25	Trevelin I	7	7	9	2.78 (0.66)	0.833 (0.213)	0.621 (0.144)
26	Trevelin II	3	3	4	2.91 (1.01)	0.812 (0.375)	0.594 (0.246)
	Argentina Atlantic						
21	Comodoro Rivadavia	6	5	5	1.75 (0.50)	0.750 (0.500)	0.375 (0.250)
	Patagonia West						
29,	LGral Carrera	5	5	6	2.36	0.542	0.446 (0.

35	I*)			(1.17)	(0.459)	363)
	Total	192	183	2.45 (0.88)	0.701 (0.383)	0.503 (0.260)

1) Population id as in map (Figure 1). Note that populations with an asterisks were merged to contain ≥ 3 infections: "Pehuenches"

merged to "San Martín de los Andes", "Los Antiguos" to "LGrál Carrera I".

2) Number of infected *B. terrestris* females sampled in population.

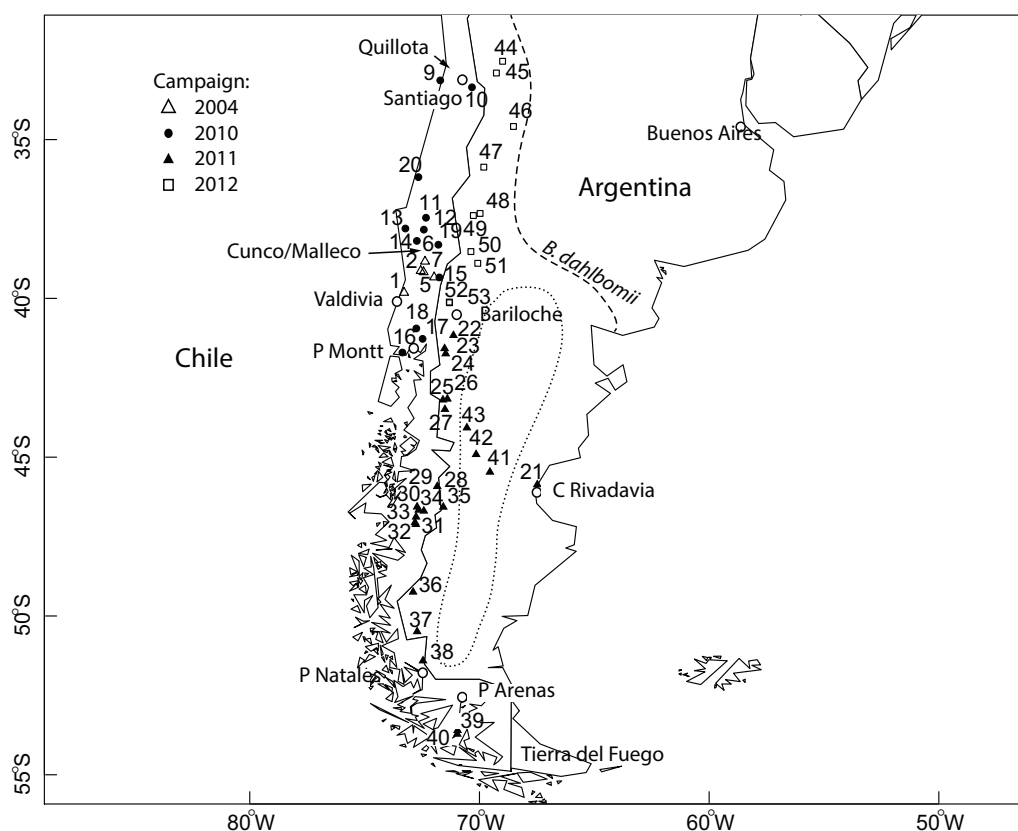
3) Number of *B. terrestris* females genotyped for this study.

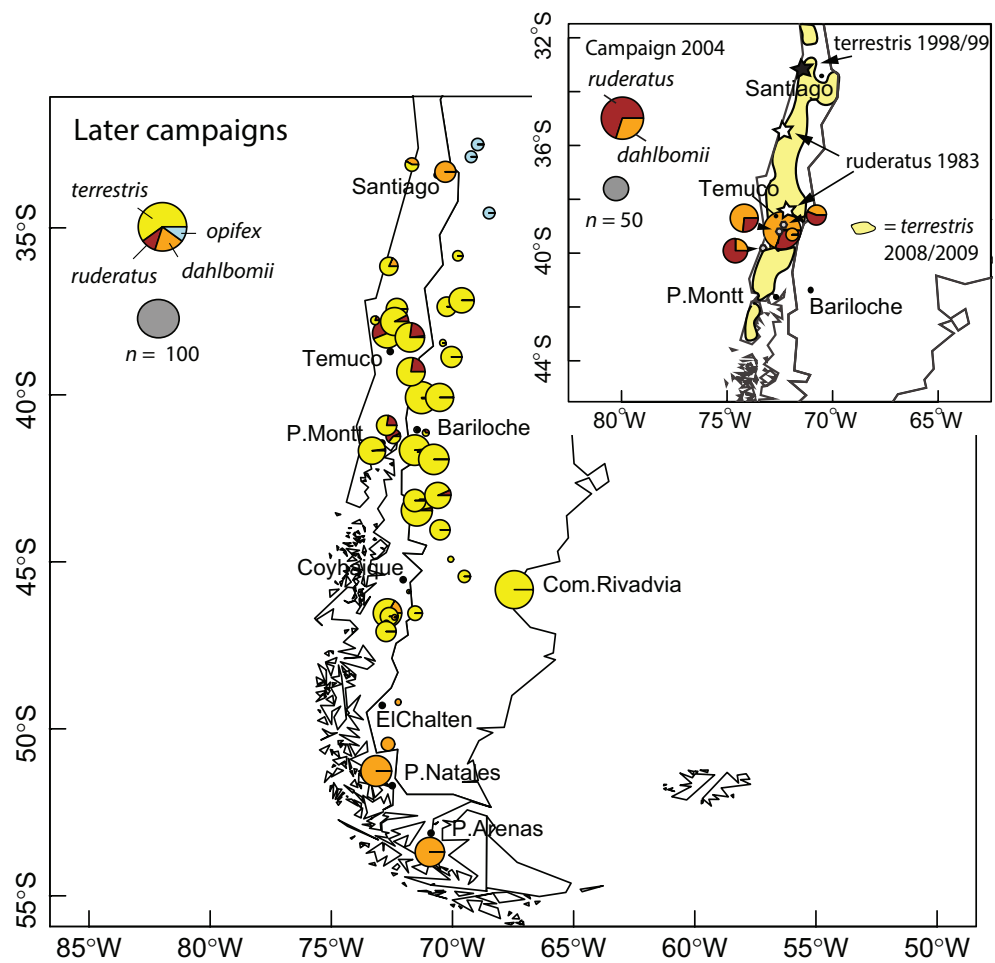
4) Number of *C. bombi* genotypes analysed (with multiple infections)

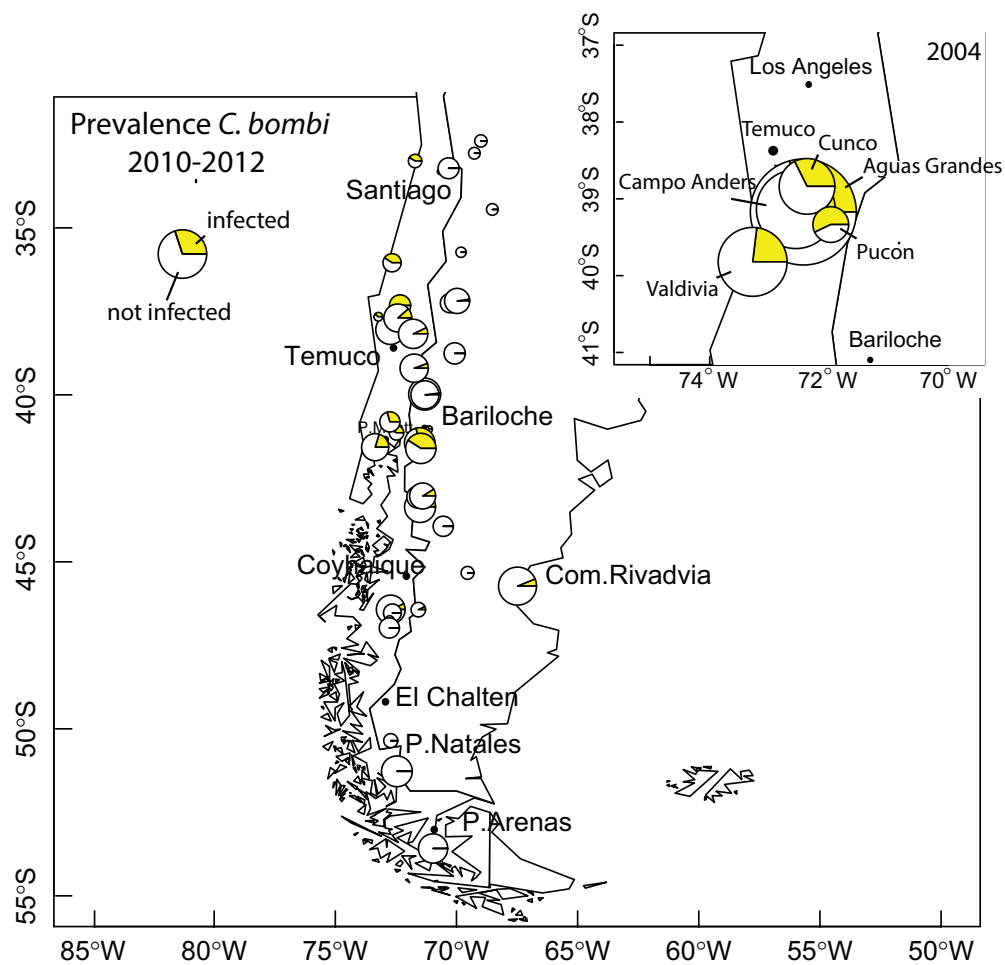
5) Average allelic richness (S.D.) over all loci; rarefaction for $n = 10$ (5 diploid individuals).

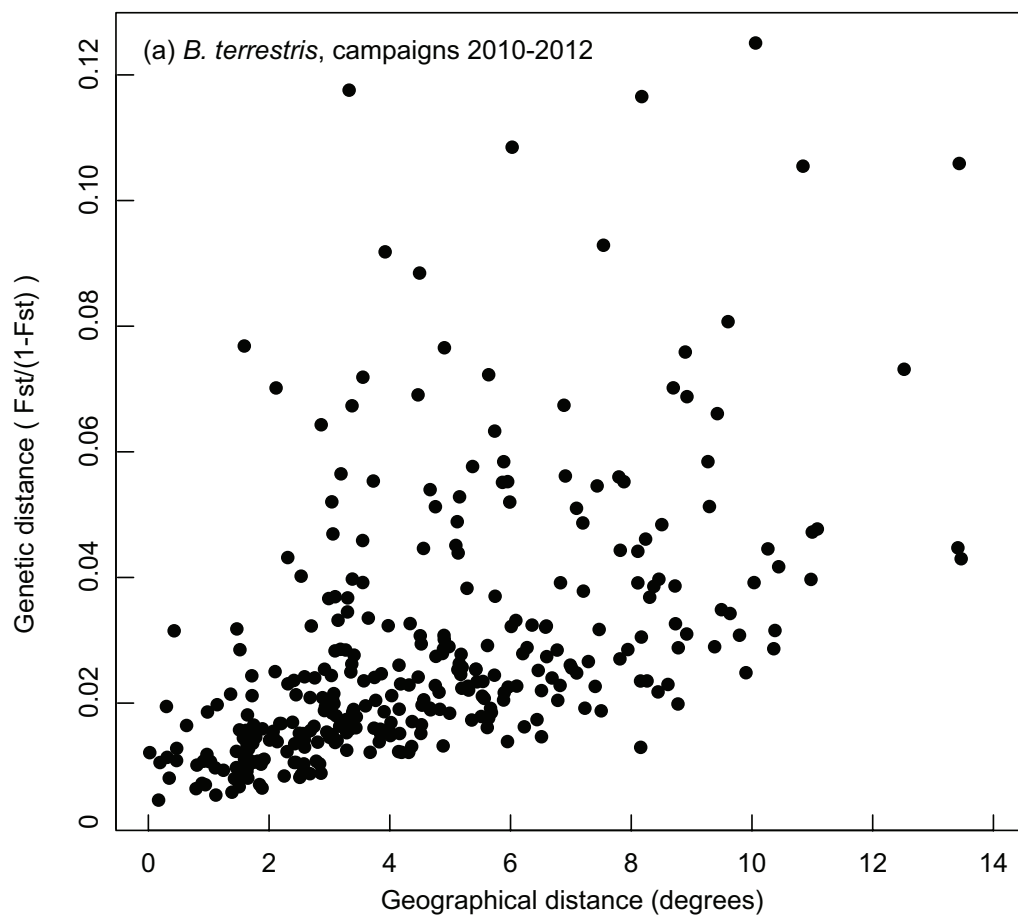
6) Hobs: fraction of heterozygotes observed over all loci, according to {Nei, 1987 #9783}.

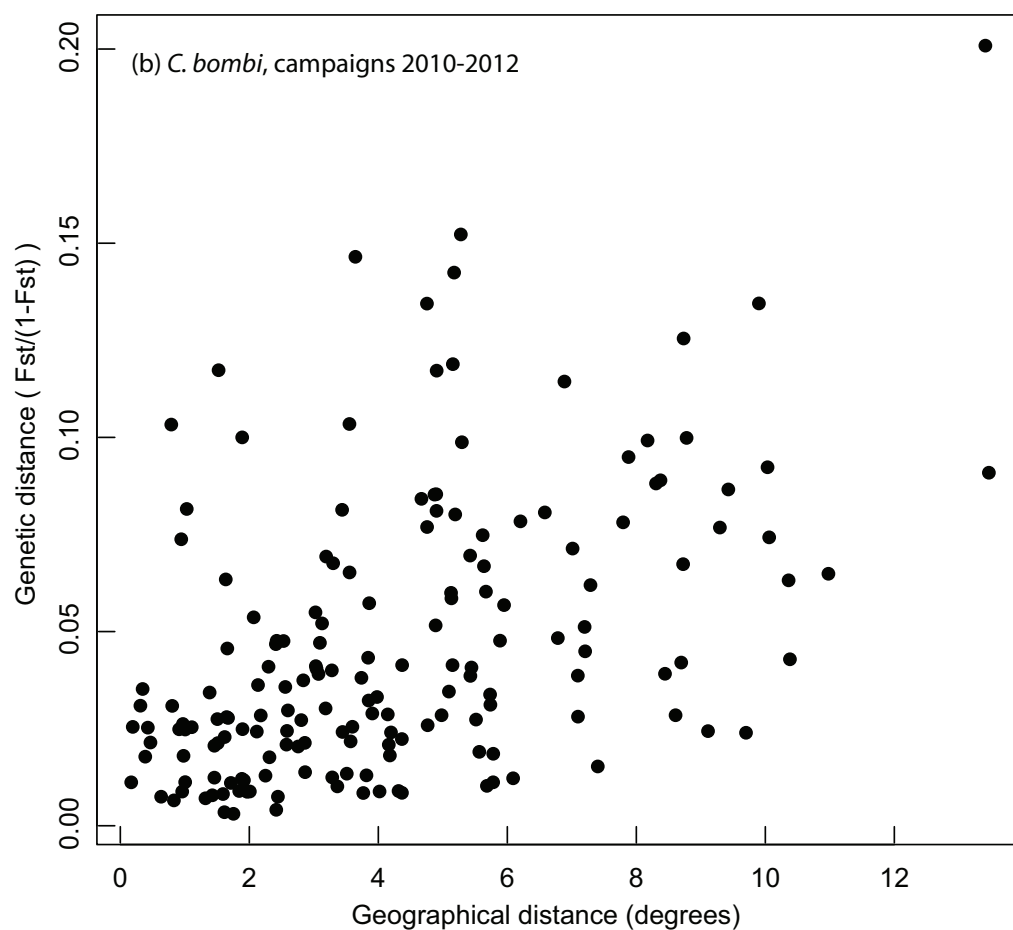
7) Hs: Genic diversity (expected heterozygosity).

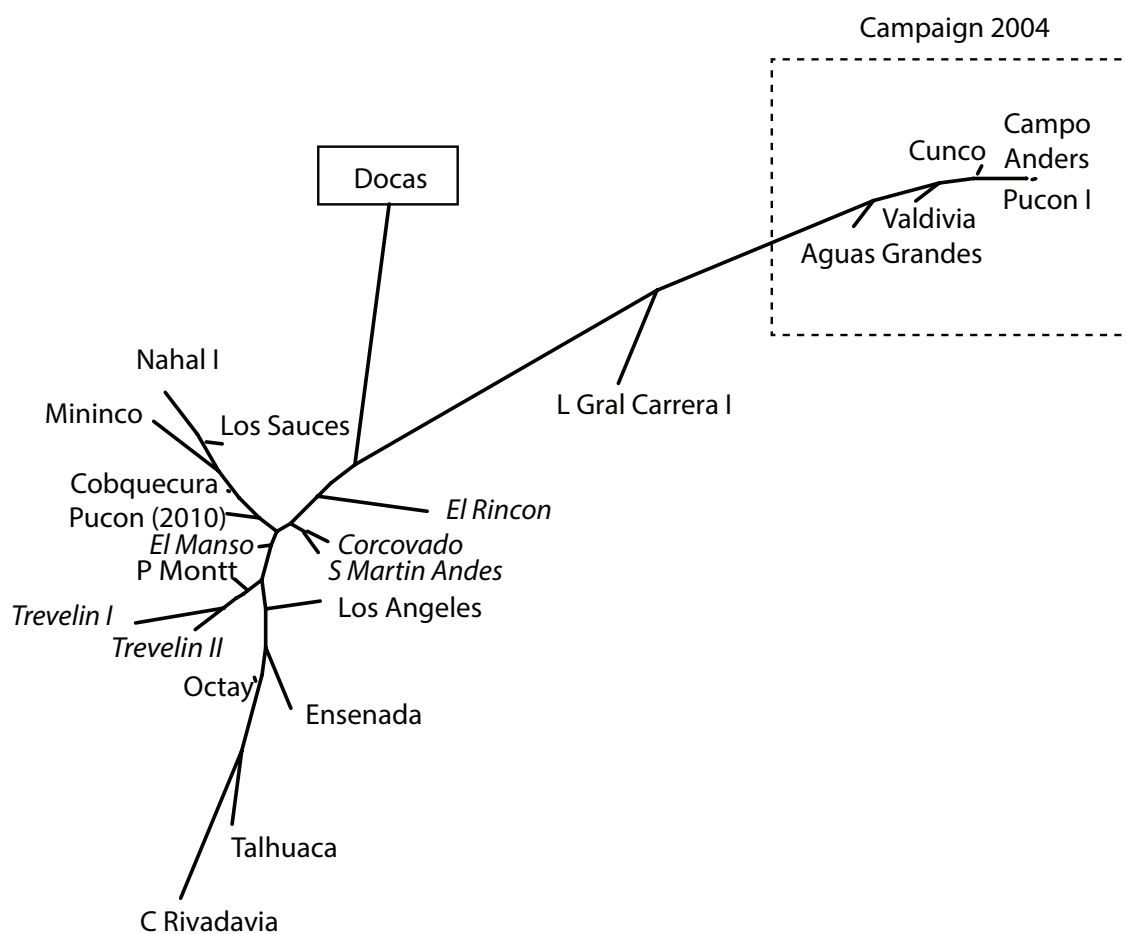


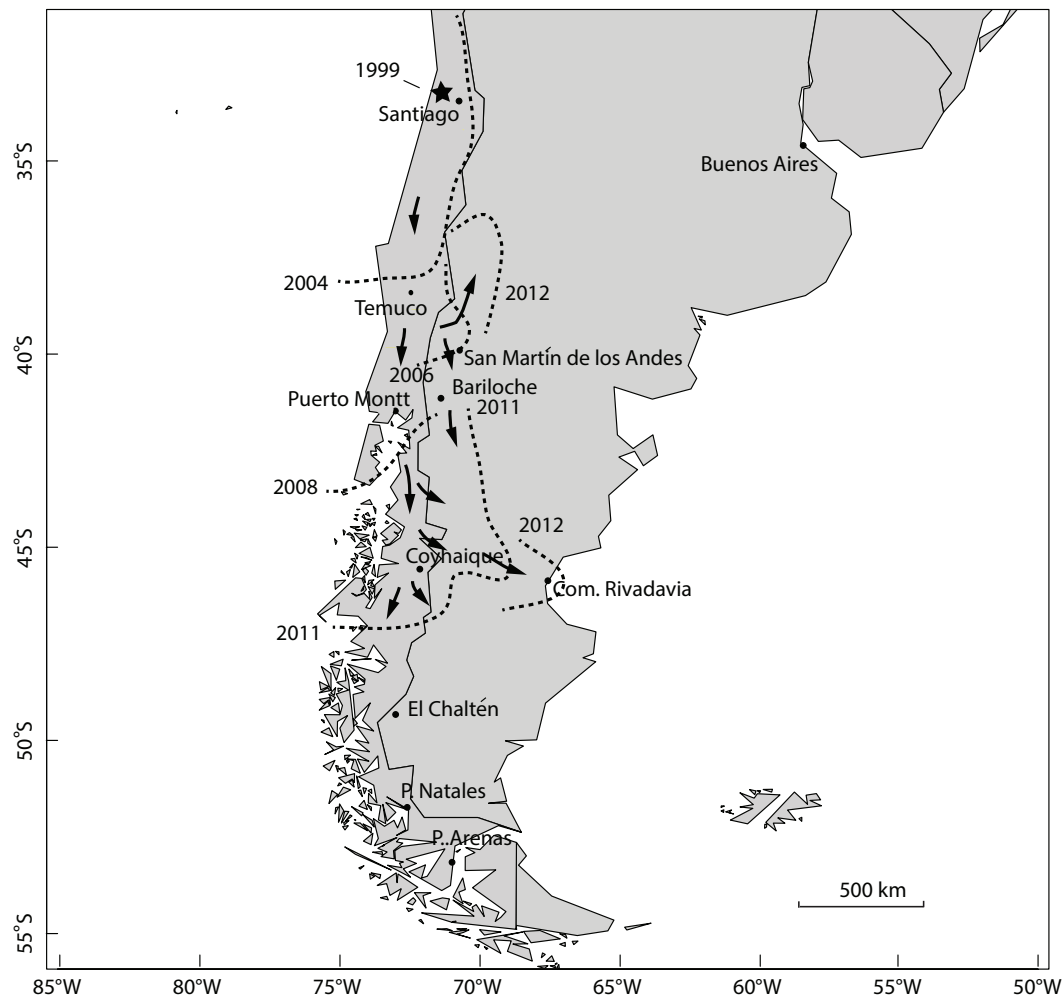












===== acceptance email =====

Dear Dr Schmid-Hempel

Re: The invasion of southern South America by imported bumblebees and associated parasites. Schmid-Hempel, Regula; Eckhardt, Michael; Goulson, David; Heinzmann, Daniel; Lange, Carlos; Plischuk, Santiago; Ruz Escudero, Luisa; Salathé, Rahel; Scriven, Jessica; Schmid-Hempel, Paul.

The above manuscript has now been accepted for publication in the Journal of Animal Ecology and will be sent to our publisher, Wiley-Blackwell. You should receive proofs from them within four weeks. Any detailed problems concerning the manuscript will now be dealt with by Wiley-Blackwell.

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Thanks again for publishing in Journal of Animal Ecology.

Yours sincerely
Professor Mike Boots

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